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Case Report

Successful treatment of a newborn with congenital hyperinsulinism having a novel heterozygous mutation in the *ABCC8* gene using subtotal pancreatectomyChi-Feng Yen^a, Chi-Yu Huang^{a,b}, Chon-In Chan^a, Chiung-Hsing Hsu^a, Nien-Lu Wang^c, Tao-Yeuan Wang^d, Chiung-Ling Lin^e, Wei-Hsin Ting^{a,b,*}^a Department of Pediatrics, MacKay Children Hospital, Taipei, Taiwan^b MacKay Junior College of Medicine, Nursing and Management, Taipei, Taiwan^c Departments of Pediatric Surgery, MacKay Memorial Hospital, Taipei, Taiwan^d Department of Pathology, MacKay Memorial Hospital, Taipei, Taiwan^e Department of Medical Research, MacKay Memorial Hospital, Taipei, Taiwan

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ABSTRACT

Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycemia in newborns and infants. CHI is characterized by unregulated secretion of insulin from pancreatic β cells. Here, we reported the case of a large-for-gestational-age, full-term newborn that suffered from CHI and developed severe and persistent hypoglycemia at an early stage of life. The infant was nearly unresponsive to medical treatment, which included continuous intravenous glucagon infusion, oral diazoxide, and nifedipine. After medical treatment had failed, an 18-fluoro L-3,4-dihydroxyphenylalanine positron emission tomography scan of the patient showed a focal lesion at the neck of the pancreas. The patient received subtotal pancreatectomy, and shortly after the procedure, the patient's blood sugar returned to the normal range. The patient was confirmed to have a novel heterozygous mutation at position c.2475+1G>A of the *ABCC8* gene. This is the first report of a focal form of CHI in a patient in Taiwan, which had preoperatively been confirmed using 18-fluoro L-3,4-dihydroxyphenylalanine positron emission tomography.

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1. Introduction

Congenital hyperinsulinism (CHI) is a genetic disorder with clinical symptoms that can range from mild to severe hypoglycemia. This disease is also the major cause of persistent hypoglycemia among newborns and infants [1]. In the literature, nine genes have been reported to be associated with CHI, with the most common genetic causes of CHI being mutations in either *ABCC8* or *KCNJ11* [2]. Both genes are located on chromosome 11p; they encode, respectively, the sulfonylurea receptor 1 (SUR1) and Kir 6.2, both of which are subunits of the adenosine triphosphate-sensitive potassium

channel (K_{ATP} channel) found in the pancreatic β cells. Most patients with either an *ABCC8* mutation or a *KCNJ11* mutation have been found to be unresponsive to diazoxide, which is the first-line treatment for CHI [3]. In the past, those who were unresponsive to medical therapy needed to receive a near-total pancreatectomy in order to treat their severe and refractory hypoglycemia. However, remarkable progress has been made regarding the diagnosis of CHI, which has included the use of molecular diagnostic approaches and 18-fluoro L-3,4-dihydroxyphenylalanine positron emission tomography (^{18}F -DOPA PET) scanning; these procedures help distinguish between the focal and diffuse forms of CHI. The information obtained from these aids in deciding an optimal management strategy for a given patient and helps predict the outcome [4]. Additionally, the incidence rate of the focal form of CHI has been reported to be higher in Asians, which makes such progress especially important for the treatment of Asian populations [5,6]. Here, we report the case of a Taiwanese newborn with CHI who was

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found to have a genetic mutation within the *ABCC8* gene; this is the first report in the literature of this mutation. The ^{18}F -DOPA PET scan diagnosed the patient as having a focal disease and accurately localized the lesion at the neck of the pancreas. The patient's blood sugar returned to the normal range after subtotal pancreatectomy. The patient, who is now being followed up at our outpatient department, has been found to be euglycemic and is fulfilling normal developmental milestones.

2. Case report

This case describes a Taiwanese male patient who was born to a nondiabetic G1P1 mother at 38 weeks of gestational age by vaginal delivery and had a birth weight of 3660 g (> 90th percentile); the baby had Apgar scores of 9 and 10 at 1 minute and 5 minutes, respectively. During hospitalization, hypoglycemia (37 mg/dL) was detected on the 2nd day of life, and a high glucose infusion rate was required to keep his blood glucose in the normal range. Despite continuous intravenous glucose supplementation and oral feeding, his blood sugar levels still fluctuated from 21 mg/dL to 145 mg/dL. Two episodes of seizure occurred during his 3rd day of life while the patient was hypoglycemic (40 mg/dL and 37 mg/dL). At this point, the patient was treated with intramuscular phenobarbital.

An endocrine survey during hypoglycemia (serum blood glucose, 39 mg/dL) showed a normal thyroid hormone level (free T4, 1.27 ng/dL), a normal growth hormone level (10.5 ng/mL), and a normal cortisol level (11.75 $\mu\text{g/dL}$), and the patient's blood was negative for ketones. However, an inappropriately elevated serum insulin level of 29.9 $\mu\text{IU/mL}$ (normal range, 3–16 $\mu\text{IU/mL}$), an abnormal c-peptide level of 4.62 ng/mL (normal range, 1–1.5 ng/mL), an abnormal ammonia level of 104 $\mu\text{g/dL}$ (normal range, 19–60 $\mu\text{g/dL}$), and a high insulin/glucose ratio of 0.77 (> 0.4) were noted. Treatment with diazoxide was tried on the 40th day of life, and the drug was gradually increased over the following days until the maximum allowable dose of 20 mg/kg/d was reached. During this period, intermittent hypoglycemia episodes were still noted, especially when we tried to taper off the intravenous glucose infusion. Nifedipine, together with a continuous glucagon infusion, was then started on the 52nd day of life, but again no clinical improvement was observed. Since the patient was unresponsive to all available medical treatments, surgical intervention was then planned in order to treat the patient's refractory hypoglycemia.

Prior to the operation, we arranged for an ^{18}F -DOPA PET scan. This resulted in the detection of a focal lesion at the neck of the pancreas (Fig. 1). Based on this finding, the patient received a subtotal pancreatectomy on the 83rd day of life, and during surgery a nodule-like lesion of about 1 cm \times 1 cm was identified at the neck of the pancreas and removed; this nodule was compatible with the lesion found on the PET scan (Fig. 2). After the operation, the patient's blood sugar level returned to the normal range, and we were able to successfully discontinue intravenous treatment of the patient on the 93rd day of life. The patient was then discharged on the 95th day of life and has been followed up at our outpatient department; he is euglycemic and has reached his normal developmental milestones.

2.1. Detection of mutations in the *ABCC8* gene

Genomic DNA was isolated from the buffy coat of the whole blood of this patient using standard procedures. All exons and intron/exon boundaries of the *ABCC8* gene was amplified by gene-specific primers. The PCR product was purified using the ExoSAP-IT PCR cleanup reagent (USB). The amplicons used the fluorescence-labeled method with an automatic sequencer (model 3730; Applied

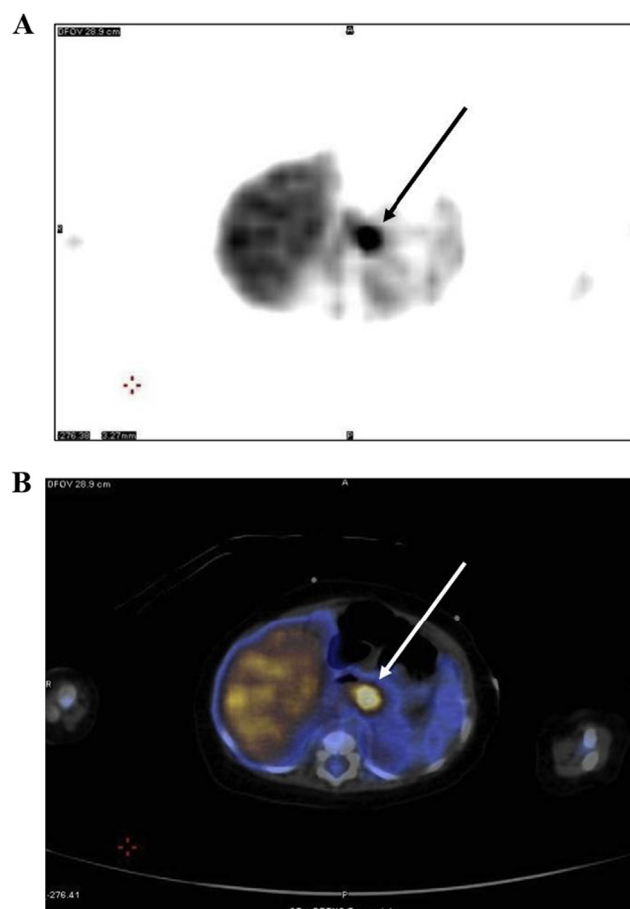


Fig. 1. (A) Transverse view of a maximum intensity projection ^{18}F -DOPA PET image that demonstrates a focal lesion at the neck of the pancreas (black arrow). (B) Transverse view of the abdominal computed tomography + PET that revealed a bright enhancement near the neck of the pancreas (white arrow). ^{18}F -DOPA PET = 18-fluoro L-3,4-dihydroxyphenylalanine positron emission tomography; PET = positron emission tomography.

Biosystems) to verify the nucleotide sequence (TaqDyeDeoxy Terminator Cycle Sequencing kit). The variations were analyzed using the reference sequences from GenBank (NM000525.3 and NM000352.4).

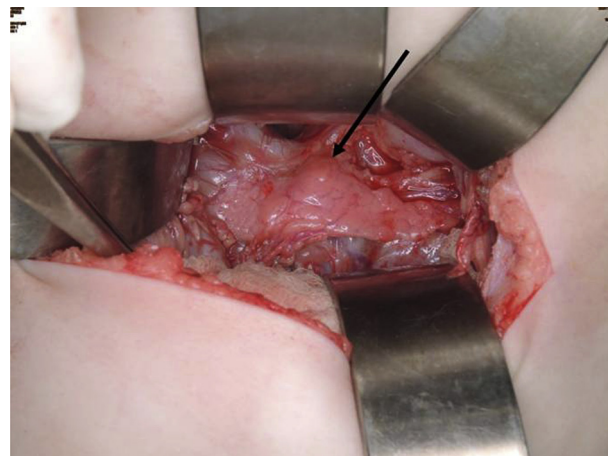


Fig. 2. A white nodule (1 \times 1 cm²) found at the neck of the pancreas during the operation.

We identified 13 heterozygous polymorphic sites in the coding region for *ABCC8* by sequencing (Table 1). We next used the splicing tool Human Splicing Finder (HSF3.0), and this predicted that one heterozygous variant, namely at position c.2475+1G>A within the intron between exons 19 and 20 of *ABCC8* (rs772026262), would most probably affect gene splicing. This is the first report of this mutation in the literature (Fig. 3). It is well known that DNA variations at the intron/exon boundaries often affect normal splicing of mRNA, which in turn affects the protein sequence and thence gene functionality.

2.2. Histological findings

Histological analysis of the pancreatic specimen showed a low-grade endocrine tumor that was composed of nests of polygonal cells with moderate to abundant eosinophilic cytoplasm (Fig. 4A). The tumor cells showed extensive immunoreactivity with antichromogranin-A antibody. Chromogranin-A is a secretory protein commonly associated with many neuroendocrine tumors (Fig. 4B).

3. Discussion

CHI is a glucose metabolism disorder that is characterized by unregulated secretion of insulin and profound hypoglycemia; it is the most common cause of persistent hypoglycemia in newborns and infants. The diagnostic criteria for CHI includes a large birth weight for gestational age, elevated glucose requirement (glucose infusion rate >10 mg/kg/min), an inappropriate elevation of insulin level, repression of blood ketone production, and an inappropriate glycemic response to glucagon when the blood sugar level is lower than 50 mg/dL [2]. Here, we reported the case of a male newborn

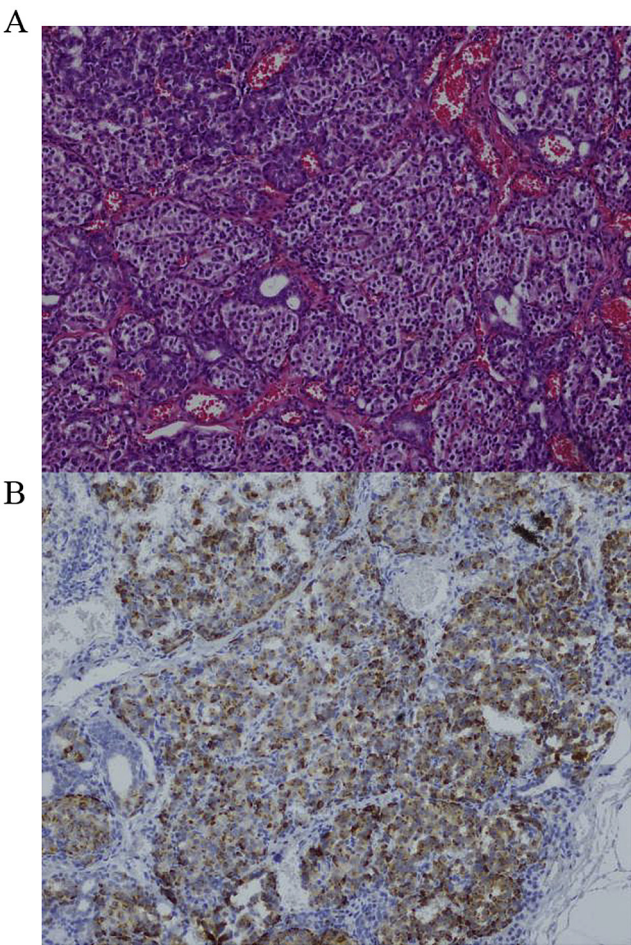


Fig. 4. (A) A pancreatic low-grade endocrine tumor was found, which was composed of nests of polygonal cells (H&E 100×). (B) The pancreatic low-grade endocrine tumor showed chromogranin-A immunostaining (100×). H&E = hematoxylin and eosin.

Table 1
Result of the screening of *ABCC8* using the splicing tool Human Splicing Finder (HSF3.0), which predicted that the variant (rs772026262, bold) most probably affected gene splicing.

SNP number	Variation description
rs2301703	c.579+14C>T
rs1799858	c.1947G>A (p.Lys649Lys)
rs10658068	c.2291+238-243del (TTTTTCT)
rs140565310	c.2291+283delA
rs1800851	c.2292-34T>C
rs1800852	c.2292-36C>T
rs772026262	c.2475+1G>A
rs3214159	c.2556+192delC
rs2106865	c.2820+17A>G
rs757110	c.4105G>T (p.Ser1369Lys)
rs4148646	c.4608+54C>G
rs41282912	c.4609-82G>A
rs1799731	c.4609-103delC

SNP: single nucleotide polymorphism.

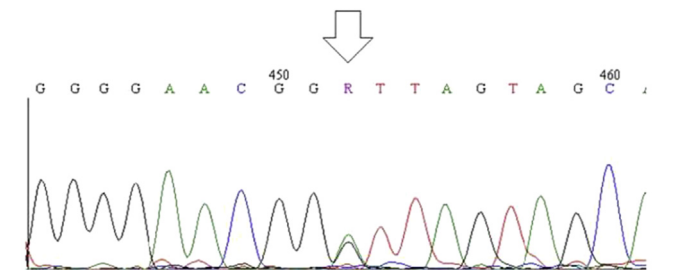


Fig. 3. A heterozygous variation at position c.2475+1G>A affecting the intron and exon boundaries between exons 19 and 20 in the *ABCC8* (rs772026262) is identified.

with CHI that presented with persistent hypoglycemia and was refractory to all medical therapies. An ¹⁸F-DOPA PET scan was able to detect a focal lesion at the neck of the pancreas, and the patient's blood sugar returned to the normal range after subtotal pancreatectomy. This was the first focal form CHI patient to be confirmed in Taiwan using a preoperative ¹⁸F-DOPA PET scan. The patient was later confirmed to have a mutation within the *ABCC8* gene, and this is the first report of this mutation in the literature.

It has been reported in the literature that CHI can be caused by mutation in nine different genes, namely, *ABCC8*, *KCNJ11*, *GCK*, *GLUD1*, *HADH*, *UCP2*, *HNF4A*, *HNF1A*, and *SLC16A1* [2,7–12]. The most common genetic causes of CHI are mutations that inactivate one of two adjacent genes that are located on chromosome 11p15.1; these are *ABCC8* and *KCNJ11*, which encode the SUR1 and Kir6.2 protein, respectively. These together form the K_{ATP} channel in pancreatic β cells [13]. Loss of K_{ATP} channel activity results in membrane depolarization, which opens the voltage-gated calcium channels and raises intracellular calcium concentrations. This then brings about an excessive and unregulated secretion of insulin even during hypoglycemia. Snider et al [3] conducted a large series of genotype–phenotype correlations involving 417 CHI cases and demonstrated that 89% of the diazoxide-unresponsive CHI carried mutations affecting the K_{ATP} channel. Those patients who were unresponsive to diazoxide then received surgery, and after surgery, they were further classified into groups

of patients with diffuse and focal CHI. Biallelic recessive K_{ATP} channel mutations were mostly associated with diffuse CHI, while the presence of a paternal monoallelic recessive mutation seemed to predict focal CHI with high sensitivity and specificity. Our patient carried a heterozygous point mutation at a splicing site within *ABCC8*, and the patient was also found to have a focal lesion affecting the neck of the pancreas. These findings are consistent with previous reports [3,14,15].

The most important recent advance in the management of children with CHI over the past 2 decades has been the introduction of ^{18}F -DOPA PET scanning to differentiate focal CHI from diffuse CHI. This advance is most important regarding Asians because the incidence rate of the focal form of CHI appears to be higher among Asian than among Caucasian children [6,16]. In many cases, focal lesions are difficult to identify during surgery and also cannot be detected by conventional image approaches such as computed tomography and magnetic resonance imaging; this is because they are generated during the normal organogenesis of the pancreas. In contrast to the diffuse disease, in which surgery is only palliative, children with focal CHI can be cured by surgical resection of the lesion; thus, it is important in this context that a preoperative ^{18}F -DOPA PET scan is usually able to accurately differentiate and localize such a lesion [17]. However, ^{18}F -DOPA PET scanning does not necessarily show the exact size of the lesion and repeated intraoperative biopsies may be necessary; these help surgeons determine the extent of pancreatectomy required [16].

Some authors have recommended that the continued presence of hypoglycemia after 5 days or more at the maximum dose of diazoxide (15 mg/kg/d) should be considered a treatment failure. At this point, these children are potential surgical candidates and should receive an ^{18}F -DOPA PET scan, which will help the surgeon establish a surgical plan [2]. In our patient, diazoxide was started on the 40th day of life, since this medicine can be used only after obtaining approval from the Taiwan Food and Drug Administration. The patient's parents preferred medical treatment to surgical intervention, which is the reason for arranging the operation only on the patient's 83rd day of life, which is 43 days after the diazoxide treatment was started. It is possible now, with the aid of an ^{18}F -DOPA PET scan, to differentiate focal CHI from diffuse CHI and accurately localize the focal lesion(s). We would advocate that ^{18}F -DOPA PET scanning is carried out earlier and that surgery to treat diazoxide-unresponsive cases is also instigated as early as possible; this will help prevent recurrent severe hypoglycemia and the possibility of a subsequent neurological deficit.

CHI is still a medical challenge for pediatric endocrinologists. Early diagnosis and adequate management are very important in order to prevent any subsequent neurological sequelae. An ^{18}F -DOPA PET scan is very helpful when trying to distinguish between the focal and diffuse forms of CHI, and the scan can also help in

accurately localizing any lesion that is present. An ^{18}F -DOPA PET scan should be considered to be a very useful guide when surgery is carried out on any CHI infant with medically uncontrollable disease.

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